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STRUCTURAL STUDIES

Polysaccharide and Lipid Composition of the Brown Seaweed *Laminaria gurjanovae*

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Abstract—Polysaccharide and lipid composition of the Pacific brown seaweed *Laminaria gurjanovae* is determined. Alginic acid is shown to be the main polysaccharide of its biomass (about 28%); it consists of mannuronic and guluronic acid residues at a ratio of 3 : 1. The yield of water-soluble polymannuronic acid is low and does not exceed 1.1% of dry biomass. High laminaran content (about 22%) is found, whereas the yield of fucoidan is no more than 3.6%. Laminaran consists of two fractions, soluble and insoluble in cold water, their ratio is 2.5 : 1. Insoluble laminaran is a practically linear 1,3-β-*D*-glucan, and the soluble fraction was shown to be 1,3;1,6-β-*D*-glucan. The oligosaccharide products of desulfation or partial acidic hydrolysis of fucoidan were studied by MALDI TOF MS; they were found to be fuco- and galactooligosaccharides. The fucoidan is suggested to be a highly sulfated partially acetylated galactofucan (Fuc/Gal is ~1 : 1). The main lipid components of the dried *L. gurjanovae* are neutral lipids and glyceroglycolipids, whereas phospholipids are found in minor amounts. The main fatty acid components of lipids are 14:0, 16:0, 16:1 ω-7, 18:1 ω-7 and 18:2 ω-6 acids.

Key words: alginate, brown seaweed, fatty acids, fucoidan, glyceroglycolipid, laminaran, Laminaria gurjanovae, phospholipid

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INTRODUCTION

Brown seaweed find practical application as a source of structurally and functionally unique polysaccharides, alginic acids and their salts (alginates), which are widely used in the food-processing industry, biotechnology, and medicine [1].[2] Other useful components of the biomass, to which belong polysaccharides of another chemical nature (laminarans and fucoidans) and low-molecular metabolites, such as mannitol, free amino acids, polyphenols, iodine-containing compounds, vitamins, and lipids [2], could also easily be obtained at a complex industrial processing of seaweed. These substances, first of all, fucoidans, are of interest mainly as biologically active compounds [3–10]. The representatives of genus *Laminaria*, some of which are widely distributed in the seas of the Russian Far East, are most convenient for practical utilization. However, their chemical compositions are non-uniformly studied. In this work, we investigated polysaccharides and lipids of seaweed *Laminaria gurjanovae*. This species inhabits depths of Sea of Japan and also Sea of Okhotsk, practically all the continental coast, and Bering Sea from Kamchatka up to Chukotka [11].

RESULTS AND DISCUSSION

We isolated polysaccharides from the seaweed *L. gurjanovae* collected from its natural populations by the following procedure. It was initially defatted with ethanol and then extracted with diluted HCl at room temperature and with hot water. The cold (Lg-E1) and hot (Lg-E2) extracts were concentrated and dialyzed, and polysaccharides were precipitated with ethanol. The yields of water-soluble fractions and their monosaccharide compositions are given in Table 1.

Table 1. Characteristics of water-soluble polysaccharide fractions obtained by a successive extraction of seaweed *L. gurjanovae*

Fraction	Yield*, %	Neutral monosaccharides, mol %			
		Glc	Fuc	Gal	Man
Lg-E1	17.6	73.2	12.5	10.1	0.4
Lg-E2	8.7	96.3	0.2	0	0

* Determined in percent of the mass of dry defatted seaweed.

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[2] Abbreviations: MTLC, micro thin layer chromatography and FAME, fatty acid methyl esters.

Table 2. Characteristics of the fractions obtained by anion exchange chromatography on DEAE-cellulose of polysaccharide preparation Lg-E1 from *L. gurjanovae*

Fraction	Eluent, [NaCl], M	Yield, %*	Content, %		*å*, kDa	Monosaccharide composition, mol %				
			SO_3Na*	polymannuronic acid, %**		Fuc	Gal	Man	Xyl	Glc
LgL	H_2O	73.1	0	0	6–25	0	0	0	0	100
LgF1	1.0	10.0	traces	7.5	20–50	31.8	9.2	40.2	5.8	0
LgF2	1.5	7.3	36.2	0	15–60	48.2	45.5	1.5	3.1	0
LgF3	2.0	2.0	38.0	0	15–60	50.0	40.6	2.0	0	0

* Percent of sample mass.

** Polymannuronic acid was precipitated by acidification of a solution of fraction LgF1 to pH 2; yield was calculated in percent of sample mass.

According to these data, Lg-E1 represents a mixture of laminaran and fucoidan, and Lg-E2, a practically pure laminaran. The remaining seaweed was treated with a $NaHCO_3$ solution, and, after concentration and dialysis, obtained sodium alginate (LgA) in 28% yield.

The alginate isolated from *L. gurjanovae* exhibited a ^{13}C NMR spectrum typical of polysaccharides of this class with the intensive signals at 101.2 (C1), 70.6 (C2), 72.6 (C3), 79.2 (C4), 77.3 (C5), and 176.1 (C6) ppm corresponding to the 1⟶4-linked residues of β-*D*-mannuronic acid and also less intensive signals at 66.0 (C2) and 80.7 (C4) ppm characteristic of the 1⟶4-linked residues of α-*L*-guluronic acid [12]. The ratio between the mannuronic and guluronic acid residues (the value of M/G approximately equal to 3) was calculated from the intensity ratio of the corresponding spectral signals. The alginic acid was polydisperse, with the interval of molecular masses been 100–300 kDa.

The fraction of water-soluble polysaccharides obtained by cold extraction (Lg-E1) was hydrolyzed to a great quantity of glucose, fucose, and galactose; a minor quantity of mannose was also found (Table 1). According to the results, fraction Lg-E1 contained laminaran consisting of glucose (about 75%), and fucoidan built up of galactose and fucose. Hydrolysis of the fraction Lg-E2 extracted with hot water practically led to the only glucose (Table 1). The results show that the seaweed is distinguished by a high content of laminaran (up to 22%), which is a prevailing component of the fraction of water-soluble polysaccharides, whereas the content of fucoidan in the biomass is relatively low. Fucoidan was readily soluble and was practically completely extracted at room temperature.

Charged polysaccharides were separated from laminaran (LgL) in the fraction Lg-E1 by a chromatography on DEAE-cellulose (Table 2). During the concentration of a water solution of LgL and cooling of the concentrate to 4°C, a formation of a precipitate was observed. This allowed us to obtain two laminaran fractions: the insoluble in cold water LgL1 and the soluble at room temperature LgL2 at the ratio of 2.5 : 1. The fraction Lg-E2 obtained by hot extraction of seaweed practically contained only laminaran (Table 1), which was separated to insoluble and soluble in cold water fractions as described above at the same ratio of 2.5 : 1.

Study of the laminaran structure has confirmed the results of one of our previous works [3]. The signals with chemical shifts of 104.1 (C1), 74.6 (C2), 86.2 (C3), 69.8 (C4), 77.3 (C5), and 62.4 (C6) ppm characteristic of 1⟶3-linked residues of β-*D*-glucopyranose [3] were present in the ^{13}C NMR spectrum of the insoluble laminaran fraction LgL1 (Fig. 1*a*). Less intensive additional signals with chemical shifts of 70.4 (C6), 76.5 (C5), and 76.2 (C5) ppm were seen in the ^{13}C NMR spectrum of the soluble laminaran fraction LgL2 along with the set of above-mentioned four intensive signals. These minor signals proved the presence of 1⟶6- and 1⟶3; 1⟶ -linked residues of β-*D*-glucopyranose, respectively (Fig. 1*b*). A minor signal with chemical shift of 64.5 ppm characteristic of mannitol is also present in the spectra of both fractions.

The molecular masses of the samples of soluble laminaran obtained by cold and hot extraction were distributed between 6 and 25 kDa with a maximum near to 10 kDa according to gel chromatography (Fig. 2). Laminarans are considered to be low-molecular polysaccharides, and their molecular masses are usually within 3–6 kDa interval [3]. According to our data, the *L. gurjanovae* laminaran has the molecular mass several times exceeding that of the laminaran from *L. cichorioides*, whose size is characteristic of laminarans [3]. However, these values contradict to the data obtained by the method of MALDI TOF mass spectrometry (Fig. 3): the molecular-mass-distribution of soluble laminaran LgL2 from *L. gurjanovae* (Fig. 3*a*) with a maximum at 4 kDa practically does not differs from that from *L. cichorioides* The only difference is that the molecules with masses up to 11 kDa are reliably registered in the mass spectrum of laminaran from *L. gurjanovae*, whereas the mass spectrum of laminaran from *L. cichorioides* reliably displays the molecules with masses of only up to 7 kDa. One can presume that the

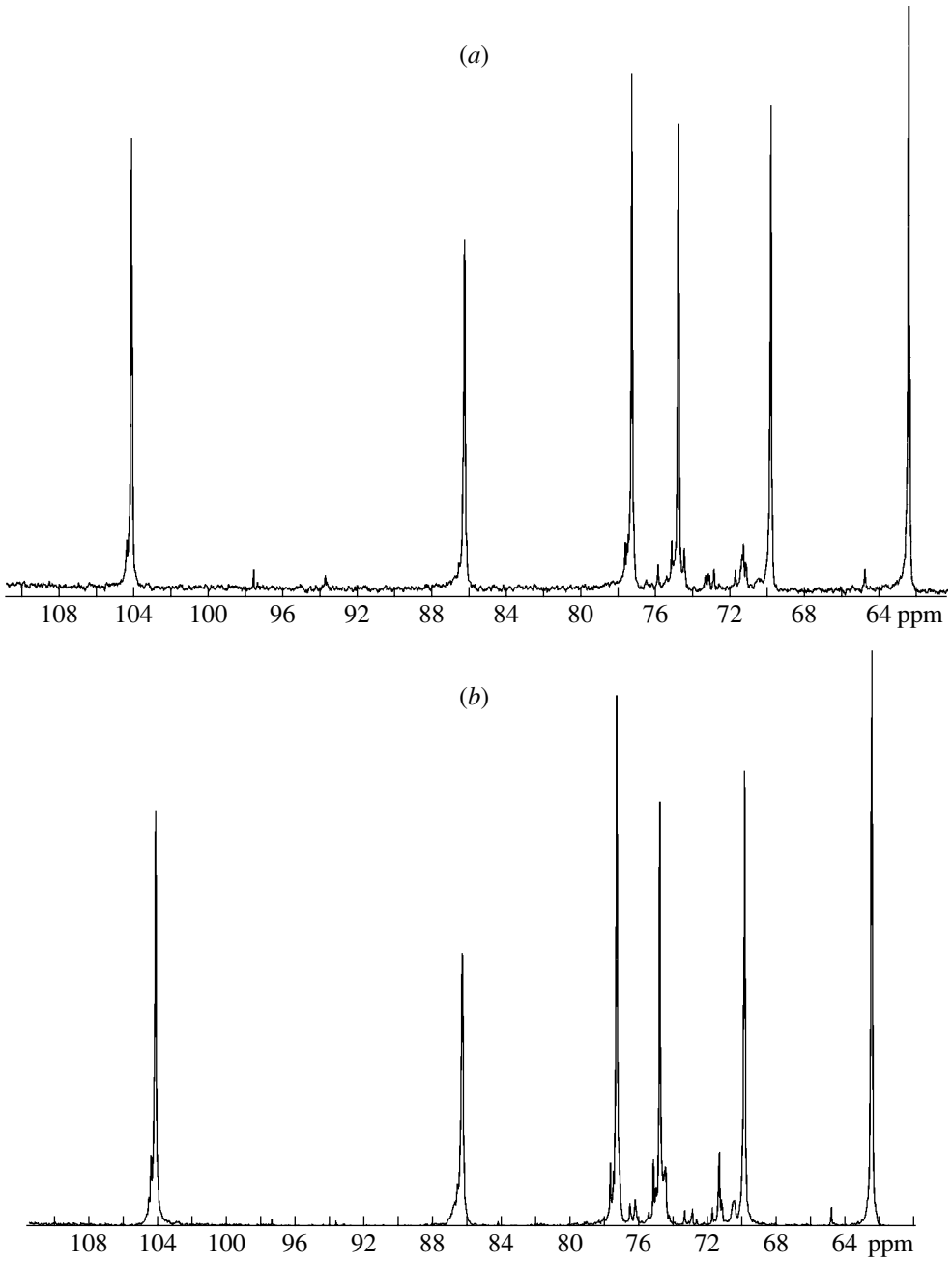


Fig. 1. ^{13}C NMR spectra of (*a*) an insoluble and (*b*) soluble laminarans from *L. gurjanovae.*

reason causing the overestimation of molecular masses of soluble laminaran registered by the gel chromatography is the predisposition of the polysaccharide molecules to association, which leads to a limited solubility of polymer in cold water and the appearance of the fraction of insoluble laminaran.

The lowly sulfated polysaccharide fraction LgF1 obtained at an anion-exchange chromatography gave several neutral monosaccharides (fucose, galactose, xylose, and mannose, Table 2) under hydrolysis. The ^{13}C NMR spectrum of this fraction exhibited signals characteristic of polymannuronic acid [8, 12], the content of which was estimated after its precipitation by acidification to pH 2.0. The yield of polymannuronic acid was 78% from the mass of the initial fraction LgF1, which is no more than 1.1% of the dry seaweed mass. The molecular mass of polymannuronic acid, determined by the method of a gel chromatography is in the range of 20–50 kDa.

The fractions LgF2 and LgF3 eluted from anion exchanger with 1.5 and 2 M sodium chloride solutions, respectively, were close in structure. Therefore, they

were combined to give a fraction of highly sulfated fucoidan (LgF) in 9.3% yield from the initial Lg-E1 extract or about 1.6% yield from the mass of defatted seaweed. The ratio Fuc/Gal in this sample was ~1 : 1 (Table 2). A characteristic absorption at 1267 cm^{-1} (S=O oscillations) is observed in the IR spectrum of fraction LgF. There are absorption bands at 851 and 828 cm^{-1}, which indicate that sulfate groups of fucoidan are in axial position at C4 and in equatorial position at C2 or C3 of the fucose and galactose residues. The values of molecular masses of LgF are within the range from 15 to 60 kDa. The ^{13}C NMR spectrum of the fucoidan LgF, like the spectra of the majority of seaweed fucoidans, exhibited a large number of poorly resolved peaks (Fig. 4). This spectrum only indicates that an appreciable amount of acetyl groups (21.7 ppm, CH_3; 175.9 ppm, C=O) are in the molecules of fucoidan, whereas the number of free hydroxy groups at galactose C6 (62.0 ppm) is low.

A sample of LgF was deacetylated and then desulfated to get a modified preparation LgF-DS. The desulfation was accompanied by a depolymerization of the fucoidan under study. The structures of the resulting oligosaccharides were analyzed by the method of MALDI TOF mass spectrometry. The peaks of ions corresponding to the mixture components consisting of fucose (Fig. 5) were found at an analysis of mass spectra. They are registered as oligosaccharide ions $[Fuc_n + Na]^+$ (n = 2–11) under the mode of positive ion registration (Fig. 5*a*) and $[Fuc_nSO_3]^-$ ions (n = 2–11) under the mode of negative ion registration (Fig. 5*b*). The presence in the spectra of signals differing from the peaks fucooligosaccharides by 16, 32, and 48 Da prove the presence of oligomers consisting of the fucose and hexose residues ($[Fuc_{n-m}Hex_m + Na]^+$; n = 3–6, m = 1–3 and $[Fuc_{n-m}Hex_mSO_3]^-$; n = 2–6, m = 1–2.

Thus, the spectral analysis showed the presence of fucooligosaccharides with a polymerization degree from 2 to 11 and mixed oligosaccharides containing fucose and galactose in their structures (e.g., Fuc_3Gal_3). The oligosaccharides containing uronic acids were absent.

The monosulfates of fucose and galactose were found in the mass spectra of the products of partial acidic hydrolysis (0.2 N TFA, 3 h, 100°C) of a sample of deacetylated fucoidan (Fig. 6). Series of ions $[Hex_nSO_3Na + Na]^+$ (n = 2–3), $[Hex_n + Na]^+$ (n = 2–5), and $[Hex_nSO_3]^-$ (n = 2–3) were also found in the spectra obtained under the corresponding modes of registration; they belong to the sulfated galactooligosaccharides with a polymerization degree from 2 to 5. Obviously, the sites of polysaccharide molecules built up of fucose residues are completely degraded under the applied hydrolysis conditions.

The fact of detection of oligosaccharides simultaneously consisting of fucose and galactose allows the presumption that the polysaccharide molecules under study contain blocks built up of the successively linked

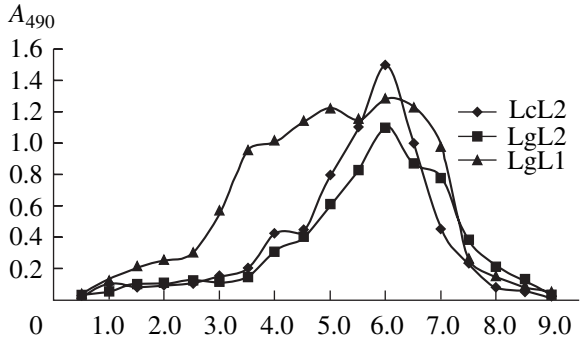


Fig. 2. Chromatography on a Superdex 75 HR 10/30 (1.5 × 30 cm) column of (LcL2) laminaran from *L. cichorioides* both (LgL1) insoluble and (LgL2) soluble laminarans from *L. gurjanovae*.

residues of fucose (up to three and, probably, more) and galactose (up to three and, probably, more).

An analysis of all the obtained information shows that fucoidan from *L. gurjanovae* is either a partially acetylated galactofucan of a block structure, in which both monosaccharide residues are sulfated, or a mixture of a partially acetylated and highly sulfated fucan, galactofucan, and, probably, galactan. It differs from similar polysaccharides, isolated from other *Laminaria* species, which are sulfated poly-(1⟶3)-α-*L*-fucans [13–15]; although, an increased content of galactose was described for fucoidans from *L. japonica* [16] and *Ecklonia kurome* [17]. At the same time, the polysaccharides in which the content of galactose is comparable with the content of fucose are known, but they had earlier been isolated from the representatives of other families. The examples are fucoidans from sporophyls *Undaria pinnatifida* [18] and *Alaria fistulosa* [19] (family Alariaceae), and also from the seaweed *Adenocystis utricularis* [20] (family Adenocystaceae). Fucoidans of various structures are of doubtless interest for a comparative study of various biological activities exhibited by them [5, 6]. The study of the structure of heteropolysaccharide from *L. gurjanovae* will be continued with the use of more effective methods of separation and with the use of chemical, physicochemical, and enzymatic methods of structural analysis.

The lipid composition was studied on the dried up and ground seaweed. It is known that drying can substantially misrepresent the results of lipid determination, first of all strongly reducing the content of polyunsaturated fatty acids up to their complete disappearance. However, the industrial processing frequently uses the preliminarily dried raw material. Therefore, the information on the lipid content of such raw material are necessary in view of the possible complex use of seaweed *L. gurjanovae*. The content of lipids extracted from the dried up seaweed was 9.2 mg/g (0.92%) of dry mass of the *L. gurjanovae* seaweed, which does not distinguish it from other species of brown seaweed, the content of the total lipids in which

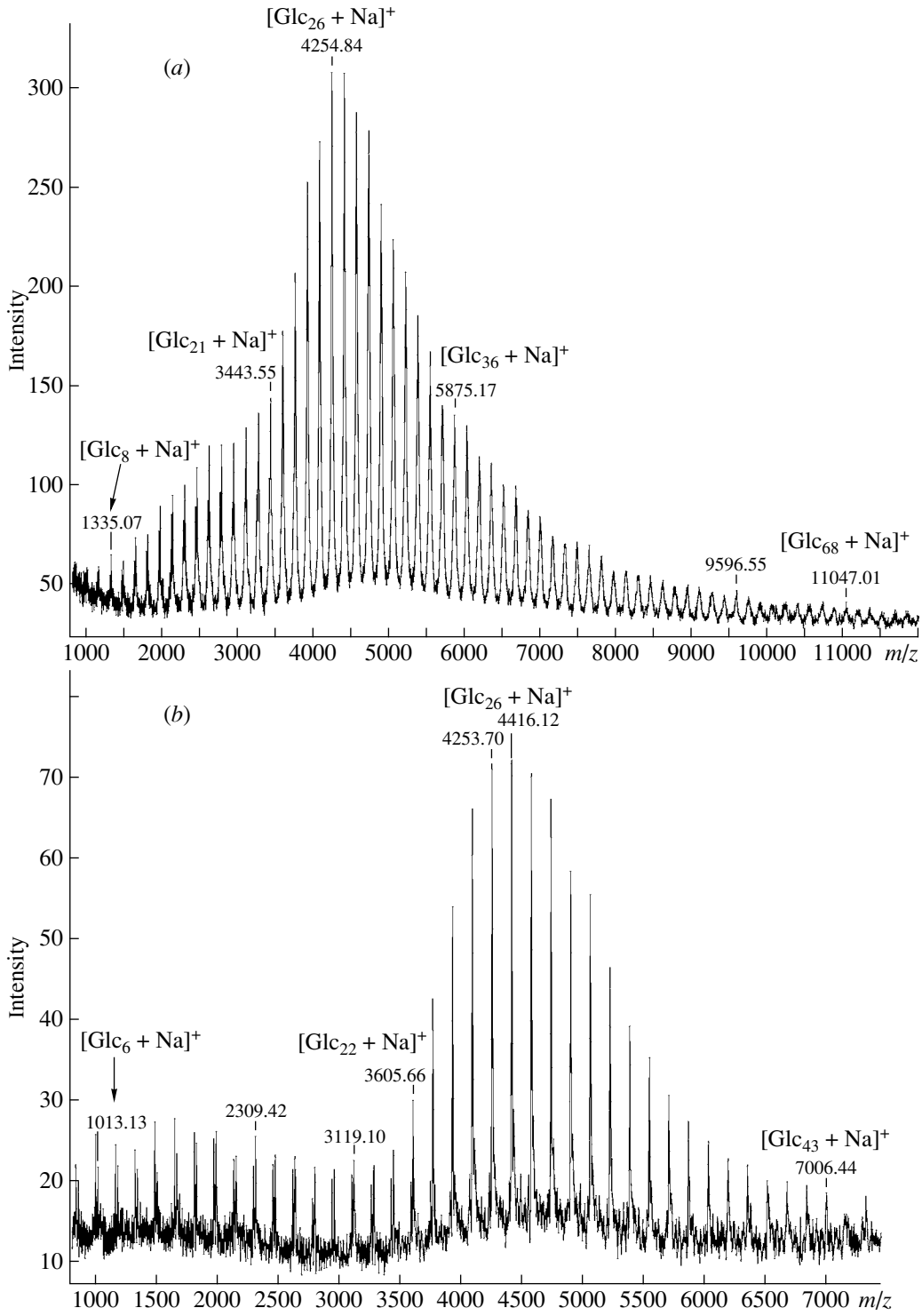


Fig. 3. MALDI TOF mass spectra of (*a*) soluble laminaran LgL2 from *L. gurjanovae* and (*b*) laminaran from *L. cichorioides*.

varies within the limits of 1.2–33.3 mg/g of dry mass [2, 21–24]. An analysis of the lipid fraction of *L. gurjanovae* showed that it is a complex mixture of neutral lipids, glyceroglycolipids, and a small amount of phospholipids. Phospholipids respond for 1.1% of total lipids or 0.01% of the seaweed dry mass, whereas glyceroglycolipids, for 12.8% of total lipids or 0.12% of the seaweed dry mass. The major part of total lipids (86.1%) are neutral lipids and pigments, extracted from seaweed together with lipids. It is necessary to mention

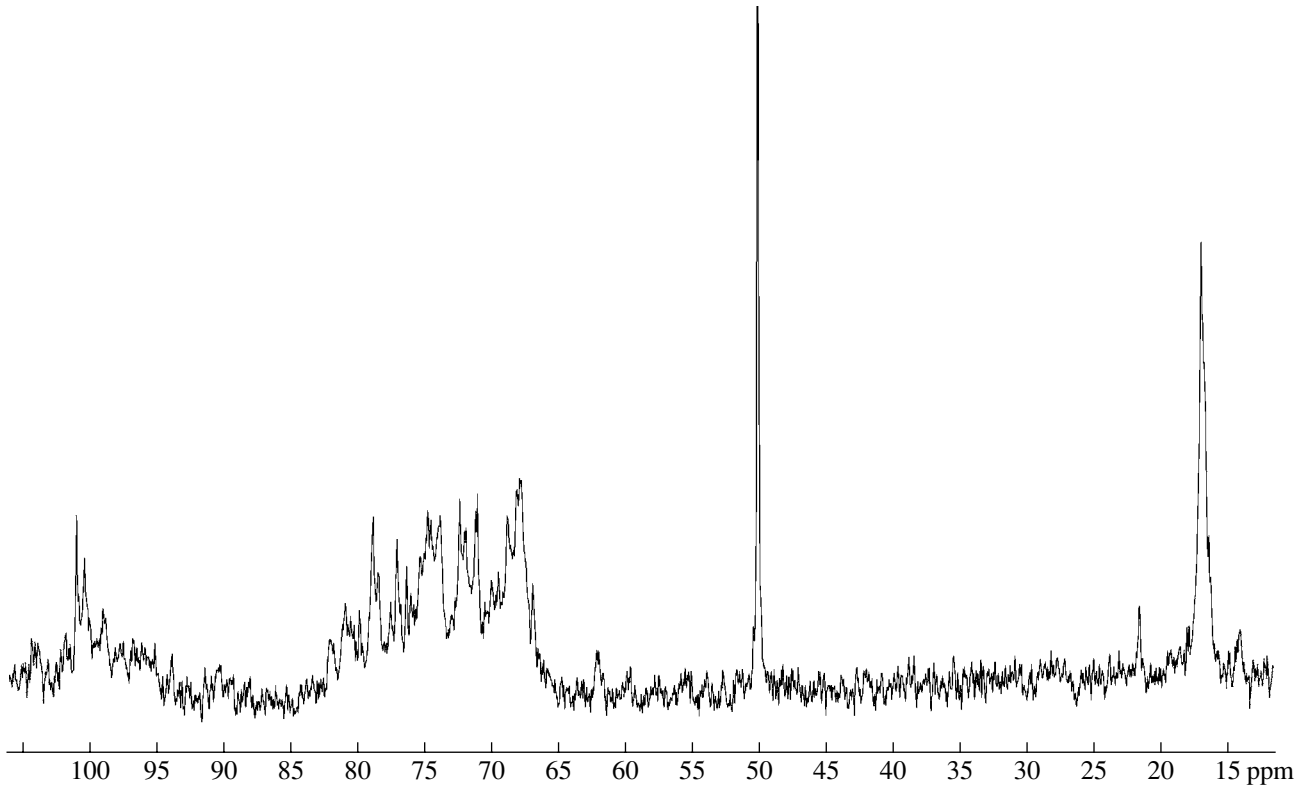


Fig. 4. ^{13}C NMR spectrum of fucoidan LgF2 from *L. gurjanovae* (the area of carboxyl group signals is not shown).

the discrepancy in the literature information on the content of total lipids and their separate classes in the brown seaweed. On the one hand, glyceroglycolipids considered as the main fraction of all the membrane lipids, with their content achieving in some species of brown seaweed of 47.2-83.1% [25, 26]. The portion of phospholipids is low in comparison with glyceroglycolipids; it varies from 2.6 to 24.5% of the total lipids [2, 21, 25, 27]. Some authors [23–25] think that neutral are the main lipids of brown seaweed; however, others show that the content of polar lipids is much more than that of apolar [27]. Most likely, this contradiction reflects the species differences; its can also be related with age, the growth stage, the season, and the place of seaweed collection [2, 22–24].

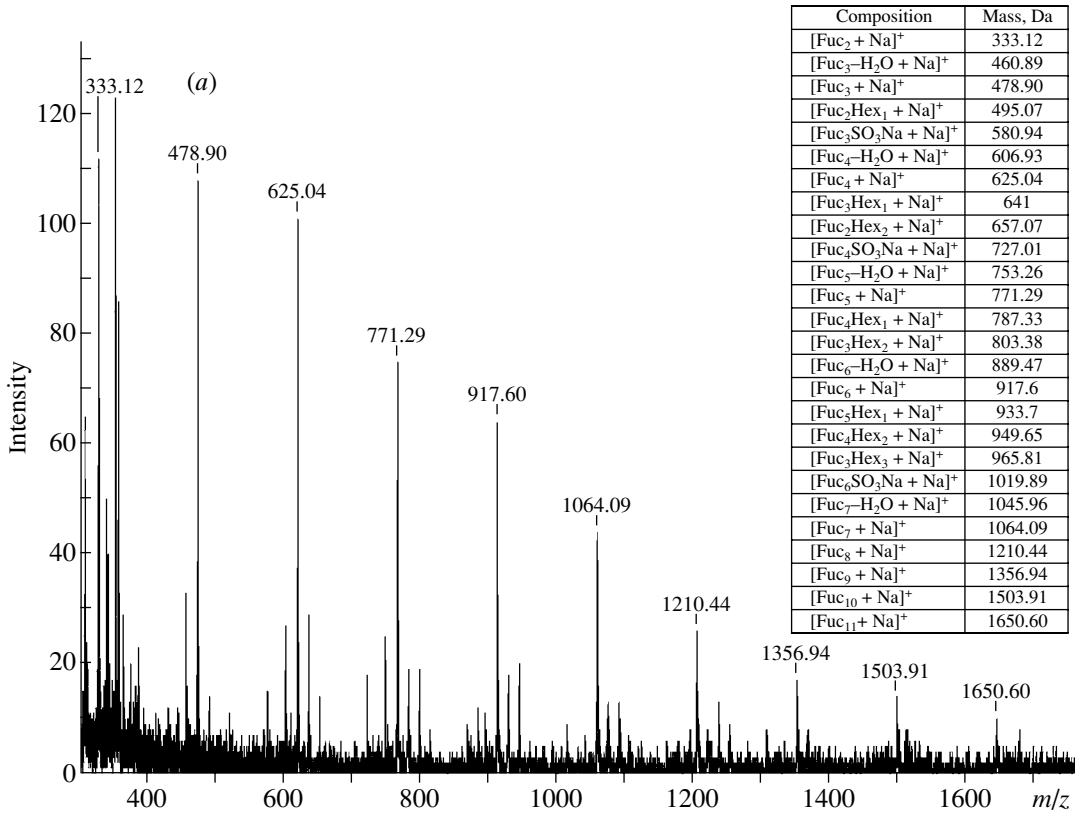
The fraction of neutral lipids of *L. gurjanovae* contains triglycerides, two unidentified lipids in the zone higher than that of triglycerides MTLC, free fatty acids, phytosterols, and diglycerides.

The pigments of *L. gurjanovae* and other laminaria species (*L. cichorioides* and *L. japonica*, our unpublished data), are represented by chlorophylls and a carotenoid substance, which gives on chromatogram one orange band at the level of fucoxanthin as described in the Experimental section.

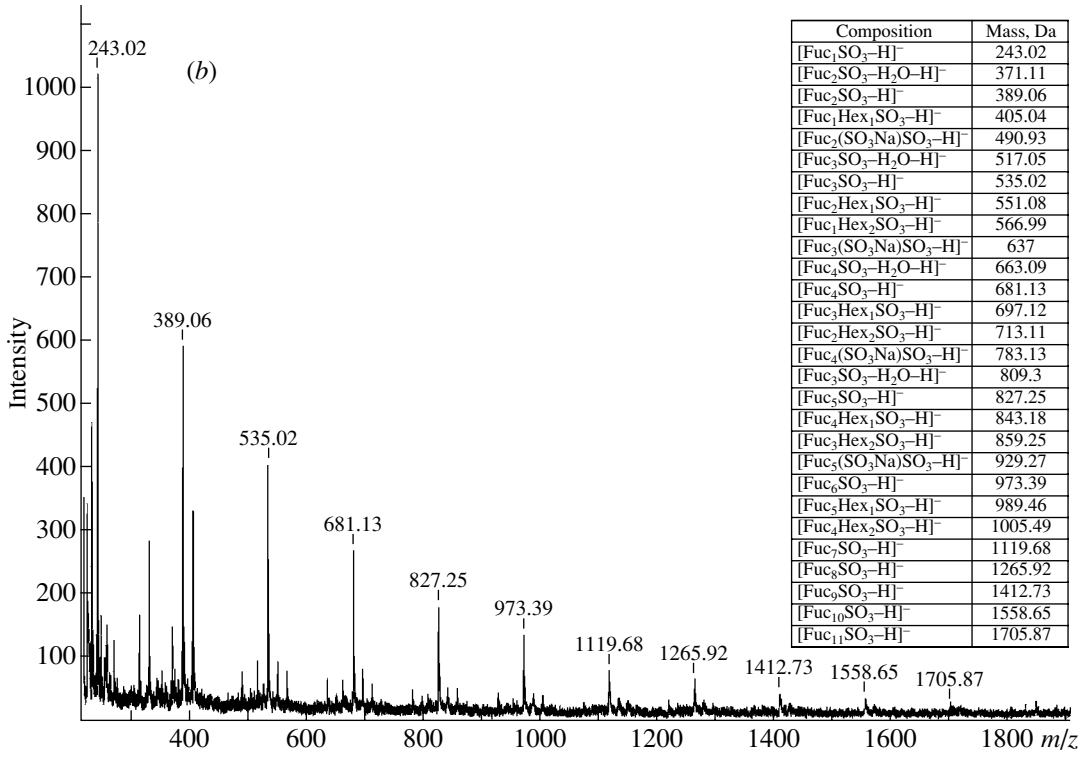
Phospholipids of *L. gurjanovae* are represented by phosphatidylcholin, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylglycerol, which does not distinguish this alga from other laminaria [2, 22]; whereas the content of phosphatidic acid is low. A significant amount of phospholipid lysoforms (phosphatidylcholin and phosphatidylethanolamine) was found, which is most likely connected with the work with dried seaweed and the storage of material for a year.

Glyceroglycolipids are represented by sulfoquinovosyldiacylglycerol, digalactosyldiacylglycerol, and monogalactosyldiacylglycerol. The intensity of staining of the monogalactosyldiacylglycerol on chromatogram (under the lipid coloring with specific reagents and after spraying the chromatogram spraying with 10% sulfuric acid in ethanol with the subsequent charring of lipids at 180–200°C) is lower than the intensity of spots of other glyceroglycolipids, which indicates its lower content. This distinguishes *L. gurjanovae* from other species of laminaria (*L. japonica* and *L. cichorioides*) in which monogalactosyldiacylglycerol prevails [2]. On chromatogram, the zone of this substance was found to display an unidentified glycolipid.

The following fatty acids were found in the total lipid extract of the dried brown seaweed *L. gurjanovae* (the percent content is shown in parentheses): 14:0 (10.22), 14:1 (5.52), 15:0 (0.56), 15:1 (0.29), 16:0 (19.25), 16:1 ω-9 (0.89), 16:1 ω-7 (11.49), 16:1 ω-5 (0.15), 16:1 ω-13 *trans* (0.34), 16:2 ω-7 (1.1), 16:2 ω (0.22), phitanic acid (0.16), 17:0 (0.21), 17:1 (0.32), 18:0 (1.12), 18:1 ω-9 (0.65), 18:1 ω-7 (11.64), 18:1 ω-6



Composition	Mass, Da
$[Fuc_2 + Na]^+$	333.12
$[Fuc_3–H_2O + Na]^+$	460.89
$[Fuc_3 + Na]^+$	478.90
$[Fuc_2Hex_1 + Na]^+$	495.07
$[Fuc_3SO_3Na + Na]^+$	580.94
$[Fuc_4–H_2O + Na]^+$	606.93
$[Fuc_4 + Na]^+$	625.04
$[Fuc_3Hex_1 + Na]^+$	641
$[Fuc_2Hex_2 + Na]^+$	657.07
$[Fuc_4SO_3Na + Na]^+$	727.01
$[Fuc_5–H_2O + Na]^+$	753.26
$[Fuc_5 + Na]^+$	771.29
$[Fuc_4Hex_1 + Na]^+$	787.33
$[Fuc_3Hex_2 + Na]^+$	803.38
$[Fuc_6–H_2O + Na]^+$	889.47
$[Fuc_6 + Na]^+$	917.6
$[Fuc_5Hex_1 + Na]^+$	933.7
$[Fuc_4Hex_2 + Na]^+$	949.65
$[Fuc_3Hex_3 + Na]^+$	965.81
$[Fuc_6SO_3Na + Na]^+$	1019.89
$[Fuc_7–H_2O + Na]^+$	1045.96
$[Fuc_7 + Na]^+$	1064.09
$[Fuc_8 + Na]^+$	1210.44
$[Fuc_9 + Na]^+$	1356.94
$[Fuc_{10} + Na]^+$	1503.91
$[Fuc_{11} + Na]^+$	1650.60



Composition	Mass, Da
$[Fuc_1SO_3–H]^-$	243.02
$[Fuc_2SO_3–H_2O–H]^-$	371.11
$[Fuc_2SO_3–H]^-$	389.06
$[Fuc_1Hex_1SO_3–H]^-$	405.04
$[Fuc_2(SO_3Na)SO_3–H]^-$	490.93
$[Fuc_3SO_3–H_2O–H]^-$	517.05
$[Fuc_3SO_3–H]^-$	535.02
$[Fuc_2Hex_1SO_3–H]^-$	551.08
$[Fuc_1Hex_2SO_3–H]^-$	566.99
$[Fuc_3(SO_3Na)SO_3–H]^-$	637
$[Fuc_4SO_3–H_2O–H]^-$	663.09
$[Fuc_4SO_3–H]^-$	681.13
$[Fuc_3Hex_1SO_3–H]^-$	697.12
$[Fuc_2Hex_2SO_3–H]^-$	713.11
$[Fuc_4(SO_3Na)SO_3–H]^-$	783.13
$[Fuc_3SO_3–H_2O–H]^-$	809.3
$[Fuc_5SO_3–H]^-$	827.25
$[Fuc_4Hex_1SO_3–H]^-$	843.18
$[Fuc_3Hex_2SO_3–H]^-$	859.25
$[Fuc_5(SO_3Na)SO_3–H]^-$	929.27
$[Fuc_6SO_3–H]^-$	973.39
$[Fuc_5Hex_1SO_3–H]^-$	989.46
$[Fuc_4Hex_2SO_3–H]^-$	1005.49
$[Fuc_7SO_3–H]^-$	1119.68
$[Fuc_8SO_3–H]^-$	1265.92
$[Fuc_9SO_3–H]^-$	1412.73
$[Fuc_{10}SO_3–H]^-$	1558.65
$[Fuc_{11}SO_3–H]^-$	1705.87

Fig. 5. MALDI TOF mass spectra of desulfated fucoidan from *L. gurjanovae* (LgF2-DS) at a registration of (*a*) positive and (*b*) negative ions.

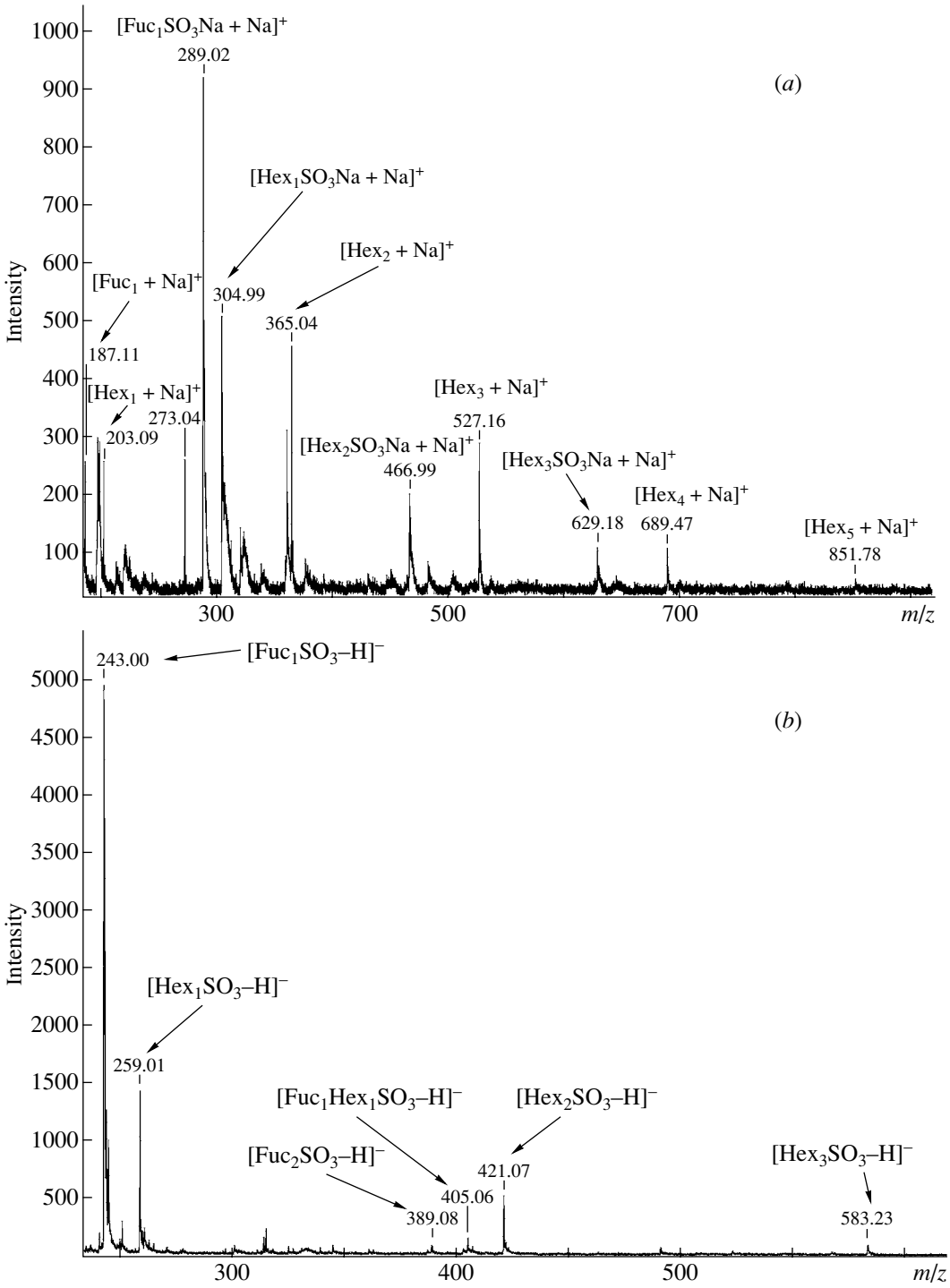


Fig. 6. MALDI TOF mass spectra of the products of partial hydrolysis of deacetylated fucoidan from *L. gurjanovae* (0.2 N TFA, 3 h, 100°C) at a registration of (*a*) positive and (*b*) negative ions.

(0.79), *x* (6.04), 18:2 ω-6 (7.43), 18:3 ω-6 (3.56), 18:3 ω-3 (2.47), 18:4 ω-3 (1.12), 18:4 ω-1 (3.0), 20:1 (1.55), 20:3 ω-6 (0.26), 20:4 ω-6 (3.42), 20:3 ω-3 (1.7), 20:4 ω-3 (0.21), 20:5 ω-3 (2.32), 22:1 ω-11 (1.16%). Monoenoic acids with 16 and 18 carbon atoms are most represented, which distinguishes this seaweed from other species of brown seaweed of the genus *Laminaria*, whose 16:0, 18:1 and polyenoic fatty acids with 18 and 20 carbon atoms are the main. The quantity of the polyenoic fatty acids in different laminaria species can achieve up to 29.1–73.1% of the total fatty acids [2], while it is much lower (about 26%) in *L. gurjanovae* An

interesting peculiarity concerns the fatty acids 20:4 and 20:5: their contents in *L. gurjanovae* are insignificant (only 3.6 and 2.3%, whereas, in other *Laminaria* species, the contents of these acids reach up to 20.8 and 19.8% [2], respectively.

The ratio of polyenoic fatty acids of the (ω-3) and (ω-6) series also appreciably differ from those in other *Laminaria* species. The content of polyenoic fatty acids of the (ω-3) series in *L. gurjanovae* is two times lower than that of fatty acids of (ω-6) series, while, in other species of *Laminaria* seaweed [2], a higher content of fatty acids of (ω-3) series has been mentioned or the quantities of the fatty acids of these two series have been described as comparable. The above-described difference in the composition of fatty acids of the total lipids of *L. gurjanovae* can be explained by various factors: conditions of habitat, time of collection, and, first of all, the procedure of preparation of raw material.

Thus, the Far-East brown seaweed *L. gurjanovae* contains great amounts of alginate and laminaran and a fucoidan of an unusual monosaccharide composition. According to the content of lipids, it is similar to related representatives of these sea plants. The wide distribution of the species makes this seaweed a prospective subject for industrial obtaining of polysaccharides, and a preliminary defatting allows the additional obtaining of extracts with a high content of lipid components, which can find application as biologically active preparations.

EXPERIMENTAL

Reagents and materials. Ethanol, acetone, butanol, chloroform, acetic acid, and inorganic salts and acids were the commercial preparations manufactured by Reakhim (Russia); the preparations of DEAE-cellulose, dextrans (molecular masses of 10, 20, 40, and 80 kDa), FAME standards, fatty acids, and cholesterol were from Sigma (United States); and commercial alginic acid, from ICN (United States). Brown seaweed *L. gurjanovae* was collected during the expedition of research vessel Academician Oparin in July, 2003 at the coast of island Big Shantar (Sea of Okhotsk). Thalluses were either treated with ethanol (1 : 1 by mass) for the subsequent isolation of polysaccharides or air-dried to constant weight and ground for the subsequent extraction of lipids. Brown seaweeds *L. cichorioides* and *U. pinnatifida* were collected in July, 2005 in the Trinity Bay (Sea of Japan) at the Sea Experimental Station of Pacific Institute of Bioorganic Chemistry (PIBOC), Far-East Branch, Russian Academy of Sciences (Primorski Krai, Hasanski raion). Laminaran was isolated from *L. cichorioides* by the method [3].

Polysaccharide extraction. A defatted seaweed *L. gurjanovae* (88 g) was stirred for 5 h in 0.1 M HCl (2×0.5 l) at room temperature, and the extracts were combined. The remaining seaweed was then extracted with water (0.5 l, 5 h at 60°C). The extracts were concentrated to 1/5 of volume by ultrafiltration with the use of Millipore 3 kDa membrane, and polysaccharides were precipitated with four volumes of 96% ethanol. The precipitates were washed with 96% ethanol and acetone and air-dried. The preparations of Lg-E1 (yield 15.5 g) and Lg-E2 (yield 7.7 g) resulted. After the water extraction, the remaining seaweed was stirred with 0.1 M $NaHCO_3$ (5 l) for 8 h at room temperature, the extract was separated and adjusted to pH 2.5 with 4 M HCl. The precipitated alginic acid was separated from supernatant by centrifugation (Eppendorf 5804 R centrifuge) at 15°C and 6500 rpm for 10 min. The precipitate was dissolved in 8% $NaHCO_3$, the solution was neutralized with 4 M HCl, dialyzed, and lyophilized to get the preparation LgA in yield of 24.6 g. The repeated extraction of the remaining seaweed did not lead to the obtaining of additional quantity of alginate.

Anion exchange chromatography. A solution of polysaccharide in 0.1 M NaCl (2.3 g in 50 ml) was applied onto a DEAE-cellulose column (Cl^- form, 3×21 cm) equilibrated with 0.1 M NaCl. Laminaran LgL was eluted with 0.1 M NaCl, and the column was then successively eluted with 1, 1.5, and 2 M NaCl solutions, each time until the disappearance in eluate of positive reaction for carbohydrates with phenol and a sulfuric acid. The fractions were dialyzed by ultrafiltration and lyophilized to get the polysaccharide preparations LgF1, LgF2, and LgF3 in yields of 230, 168, and 46 mg, respectively. The laminaran yield was 1.68 g.

Carbohydrate content in the isolated samples and fractions was colorimetrically determined by the reaction with phenol and a sulfuric acid [28].

The content of sulfate groups in polysaccharides was determined by titrimetry [29].

Deacetylation was carried out by the treatment of polysaccharides with ammonia inwater solution [15].

Sulfate groups were removed by solvolytic desulfation (heating of polysaccharide pyridine salts in DMSO with a methanol additive) [13]. Upon the end of reaction, the mixture was diluted with water, twice lyophilized for the removal of DMSO, and the residue was used for the analysis by MALDI TOF mass spectrometry.

Monosaccharide composition was determined on a Biotronik IC-5000 carbohydrate analyzer (Germany) using a Shim-pack ISA-07/S2504 (0.4×25 cm) column eluted with a potassium borate buffer at an elution rate of 0.6 ml/min. Detection was carried out by bicinchoninate method and integration, on a Shimadzu C-R2 AX system. Monosaccharides (rhamnose, ribose, mannose, fucose, galactose, xylose, and glucose) were used as standards for HPLC.

Acidic hydrolysis of polysaccharides. Solution of a substance (5 mg) in 2 N TFA (500 μl) was heated for 8 h at 100°C, and the acid was three times coevaporated with methanol on a rotary evaporator.

Partial acidic hydrolysis of polysaccharides. A solution of 5 mg deacetylated fucoidan in 0.2 N TFA

(500 μl) was heated for 3 h at 100°C, and the acid was three times coevaporated with methanol on a rotary evaporator.

Molecular mass was determined the method of gel chromatography on a Superdex 75 HR 10/30 or Superose 6 HR 10/30 column (1.5 × 30 cm, American Pharmacia Biotech AB). Elution was carried out with 0.05 M phosphate buffer, pH 7.2, containing 0.15 M NaCl. The elution rate was 0.2 ml/min. Fractions (0.5 ml) were analyzed for carbohydrates by the reaction with phenol and a sulfuric acid. Dextranes with *M* 10, 20, 40 and 80 kDa for Superdex 75 HR 10/30 and with *M* 40, 80, and 200–300 kDa for Superose 6 HR 10/30 were used as standards.

IR spectra of polysaccharides were registered in KBr pellets on a Carl Zeiss IR-75 spectrometer.

Spectra of ^{13}C NMR for solutions of substances in D_2O were obtained on a Bruker Avance DPX-300 NMR spectrometer with working frequency of 75.5 MHz at 60°C.

MALDI TOF mass spectra were measured on a Biflex III Bruker mass spectrometer (Germany) equipped with a nitrogen laser (337 nm) at an accelerating voltage of 19 kV. 2,5-Dihydroxybenzoic acid (50% solution, 10 mg/ml of 1 : 1 acetonitrile–water) was used as a matrix in a mode of positive ion registration. At a mode of negative ion registration, a solution of *D-erythro*-pentose phenylozazone (arabinoozazone, 10 mg/ml in methanol [30] was used as a matrix. A water solution of a substance under study (1 mg/ml) was applied onto a target in a volume of 1 μl and, then, applied a matrix (1 μl) and dried in an air stream. Mass spectra were obtained with the use of external calibration by the peaks of matrix and angiotensin-II (Sigma).

Extraction of lipids. Total lipids were successively extracted from a dried and ground seaweed *L. gurjanovae* with acetone (at 1 : 1 biomass–acetone ratio, hereinafter, volumetric ratios are given), 1 : 1 chloroform–acetone mixture, and three times with 1 : 1 chloroform–ethanol mixture. The extracts were combined, evaporated to 1/3 of initial volume, and an equal volume of chloroform was added to the residue. The resulting mixture was five times washed with water. The lipid extract was evaporated on a rotary evaporator to dryness, dissolved in a small volume of chloroform and stored at –20°C.

The content and composition of lipids.. The total lipids were determined in extracts by weighting, phospholipids, by the phosphorus content [31], and glyceroglycolipids, by the content of galactose [32]. MTLC of phospholipids was carried out by the method [33]. Phospholipids were detected on plates by molybdate reagent [31] and by spraying with 10% H_2SO_4 in alcohol and subsequent heating at 180–200°C. Neutral lipids were separated as methyl esters by MTLC in 80 : 20 : 1 hexane–diethyl ether–AcOH system in the presence of FAME standards, cholesterol, triglycerides, and free fatty acids. Neutral lipids were detected by spraying with 10% ethanolic H_2SO_4, followed by heating at180–200°C. The qualitative composition of glyceroglycolipids was determined by MTLC in 91 : 30 : 8 acetone–benzene–water system with the detection by orcinol reagent [34]. FAME of the total lipids of *L. gurjanovae* were obtained by the method [35] and purified by preparative MTLC in 95 : 5 hehane–diethyl ether system. The FAME zone was removed from a plate, transferred on a filter, and eluted with a 1 : 1 chloroform–methanol mixture. The eluate was evaporated in a vacuum to dryness, the residue was dissolved in hexane and used for the determination of FAME composition on gas chromatograph Shimadzu 17A equipped with a flame-ionization detector and a capillary column (0.25 mm × 50 m) with a thin layer of Supelco Wax 10M liquid phase. Evaporator temperature was 240°C and column temperature, 210°C; helium was used as the carrier gas (50 ml/min). FAMEs were identified by a comparison of their retention times with standards and by the values of carbon numbers [36].

Pigments. Pigments were separated by MTLC in 50 : 10 : 5 : 0.1 benzene–ethyl CH_3COOH system in the presence of standard fucoxanthin, we isolated by column chromatography on silica gel L 100–160 μ m (Chemapol, Lachema) from brown seaweed *U. pinnatifida* in solvents systems hexane–acetone (9 : 1 and 8 : 2).

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